

CHEMISCREEN™ MEMBRANE PREPARATION HUMAN RECOMBINANT 5-HT₆ SEROTONIN RECEPTOR

CATALOG NUMBER:	HTS111M	QUANTITY:	200 units
LOT NUMBER:	RI08010045	VOLUME/CONCENTRATION PER VIAL:	1 mL, 1 mg/mL

BACKGROUND: The neurotransmitter serotonin/5-hydroxytryptamine (5-HT) regulates a wide variety of neurological functions. A family of 13 receptors (12 GPCRs and one ion channel) mediate the effects of serotonin. The serotonin receptor 5-HT₆ is a G_s coupled receptor expressed solely in the CNS, primarily in the limbic and cortical regions. 5-HT₆ appears to play a role in memory and learning, obesity, psychosis, anxiety and epilepsy (Woolley *et al.*, 2004; Fisas *et al.*, 2006). In particular, a 5-HT₆-selective agonist caused significant weight loss in a rat model of diet-induced obesity. Millipore's 5-HT₆ membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression; thus, they are ideal HTS tools for screening of agonists and antagonists of 5-HT₆. The membrane preparations exhibit a K_d of 0.44 nM for [¹²⁵I]-SB258585. With 0.25nM [¹²⁵I]-SB258585, 5µg/well 5-HT₆ Membrane Prep typically yields greater than 6-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay and GTPγS binding.

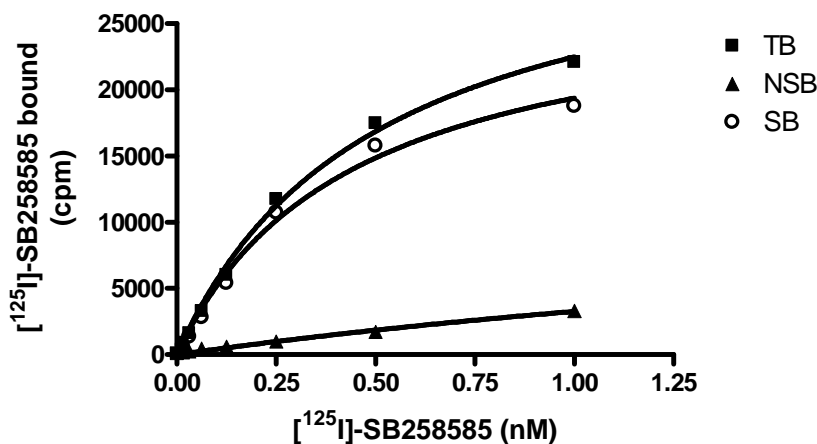


Figure 1. Saturation binding for 5-HT₆. 5 µg/well 5-HT₆ Membrane Preparation was incubated with increasing amount of ¹²⁵I-labeled SB-258585 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled 5-HT. Specific binding (SB) was determined by subtracting NSB from TB.

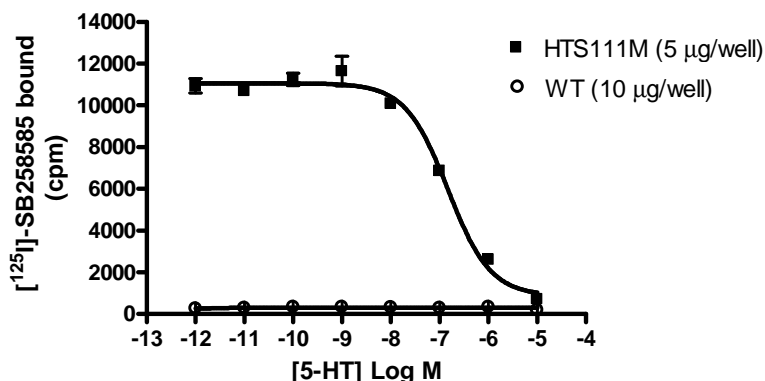


Figure 2. Competition binding for 5-HT₆. 5-HT₆ Membrane Preparation (5 µg/well) and wild-type Chem-1 Membrane Preparation (10 µg/well; Millipore catalog # HTS000MC1) were incubated in a 96-well plate with 0.25 nM ¹²⁵I-labeled SB258585 and increasing concentrations of unlabeled 5-HT. More than 6-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of 5-HT₆ Receptor membrane prep.

	5 µg/well	10 µg/well
Signal:background	12.8	12.3
Specific binding (cpm)	10192	12675

SPECIFICATIONS: 1 unit = 5 µg
 B_{max} for [¹²⁵I]-SB258585 binding: 6.1 pmol/mg protein
 K_d for [¹²⁵I]-SB258585 binding: ~0.44 nM

TRANSFECTION: Full-length human HTR6 cDNA encoding the 5-HT₆ Serotonin Receptor (Accession Number: NM_000871)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous 5-HT₆ expression.

RECOMMENDED ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, an FC 96-well harvest plate (Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with the binding buffer. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 20 mM HEPES, 3 mM MgCl₂, 2 mM Ascorbic acid, pH 7.4, filtered and stored at 4°C

Radioligand: [¹²⁵I]-SB258585 (Perkin Elmer #:NEX424)

Wash Buffer: same as the binding buffer.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 6-fold signal:background with ¹²⁵I labeled SB258585 at 0.25 nM

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.

Packaging method: Membranes protein were adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING:

Maintain frozen at -70°C up to expiration date indicated on the label. Do not freeze and thaw.

REFERENCES:

Woolley ML *et al.* (2004) 5-HT₆ receptors. *Curr. Drug Targets CNS Neurol. Disord.* 3: 59-79.

Fisas A *et al.* (2006) Chronic 5-HT₆ receptor modulation by E-6837 induces hypophagia and sustained weight loss in diet-induced obese rats. *Br. J. Pharmacol.* 148: 973-83.

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